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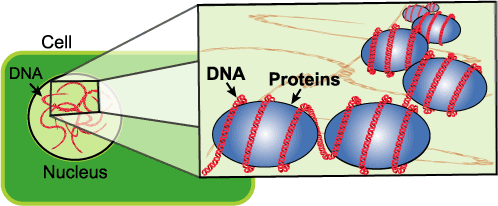
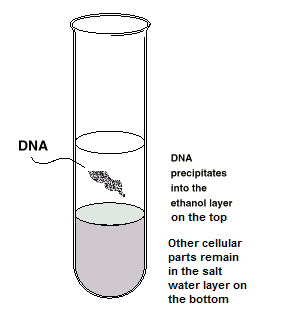
**Unit 6, Part 4 Notes: Genetic Technology**

Pre-AP Biology, Mrs. Krouse

**What is genetic technology?**

Genetic technology is used to remove DNA from cells, study it, and alter it. When scientists alter DNA and put it into an organism’s cells, this is known more specifically as genetic engineering.

**How can DNA be removed from cells?**

1. Removing DNA from cells so that it can be studied and modified is called DNA extraction. We did this in our strawberry and banana DNA extraction lab. The specific procedure for extracting DNA differs a bit based on the cell type and various other factors, but it typically involves the following steps.
2. Chemicals called detergents are used to break down lipids from the cell membrane and nuclear membrane. Dish soap contains detergents, so it is often used in high school DNA extraction labs.
3. Enzymes called proteases are used to break down proteins in the cell, including the histone proteins that DNA wraps around in eukaryotic cells (see image below). Meat tenderizer powder (used in cooking to make steaks more tender) contains two protease enzymes—bromelain (found in pineapple juice) and papain (found in papaya juice). As such, meat tenderizer powder is often used in high school DNA extraction labs.
4. 
5. **A concentrated salt solution (i.e., salt water) is used to separate DNA from the other components of the cell (ex: proteins and lipids). The salt causes the DNA to begin clumping together.
6. After this, cold alcohol (either ethyl alcohol or isopropyl alcohol) is poured over the salt solution. DNA moves up alcohol layer and further clumps together. By clumping together, the DNA forms a “precipitate,” which is a solid that forms in a liquid solution. At this point, the DNA can be removed from the alcohol layer and studied.
   1. *\*\*\*Note: In step #4, the other cellular components (ex: proteins and lipids) stay in the salt water layer at the bottom of the test tube or beaker\*\*\**

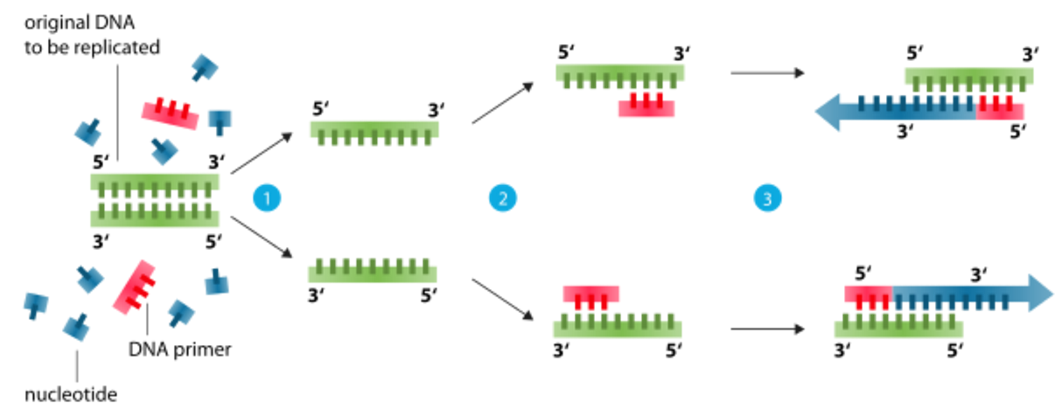
**How can DNA be studied?**

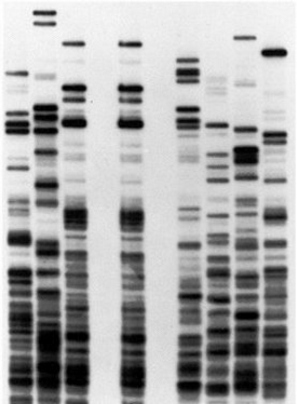
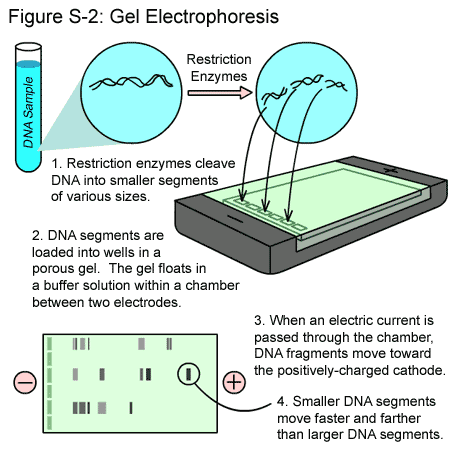
1. Typically, before scientists study a particular DNA sequence, they must make copies of that sequence. This is often referred to as “amplification” of the DNA sequence. One method to make these copies is called Polymerase Chain Reaction (or PCR for short). Scientists have to extract DNA from cells to use during PCR. PCR involves three main steps, which are listed below…

A. High heat is used to break hydrogen bonds between complementary bases on the two DNA strands. This separates the DNA strands just like helicase does during replication inside cells. Scientists often say that this step involves “denaturing” the DNA. This is step #1 in the image below.

B. Scientists mix the denatured DNA with specific primers, which are short chains of nitrogen bases (A, T, C, and G) that are complementary to the “target sequence” on the DNA, which is the sequence that they want to copy. The primers attach to the beginning of the target sequence. This is step #2 on the image below.

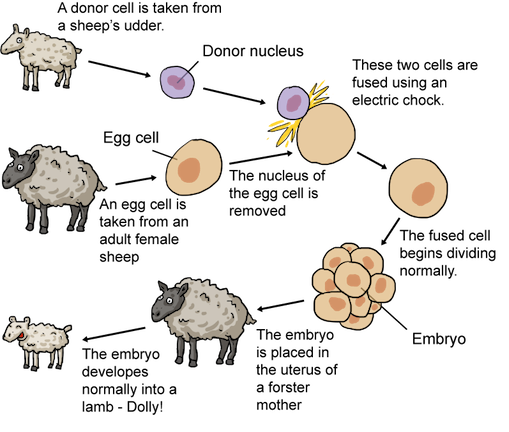
C. DNA polymerase taken from a species of bacterium called *Thermus aquaticus* is used to copy the target sequence beginning at the primers. This type of DNA polymerase is called Taq polymerase (named after the species it comes from). Scientists use Taq polymerase because *Thermus aquaticus* normally lives in hot springs and hydrothermal vents, so its proteins (including the enzyme DNA polymerase) function properly at high temperatures. They do not denature at the high temperatures we need to use during PCR to separate the DNA strands. Taq polymerase works in the same way that our DNA polymerase works. It gathers free nucleotides and attaches them to complementary nucleotides on the template DNA strand. It reads from the 3’ end towards the 5’ end of the template DNA, so it builds the new DNA from its 5’ end towards its 3’ end. This is step #3 on the image below.



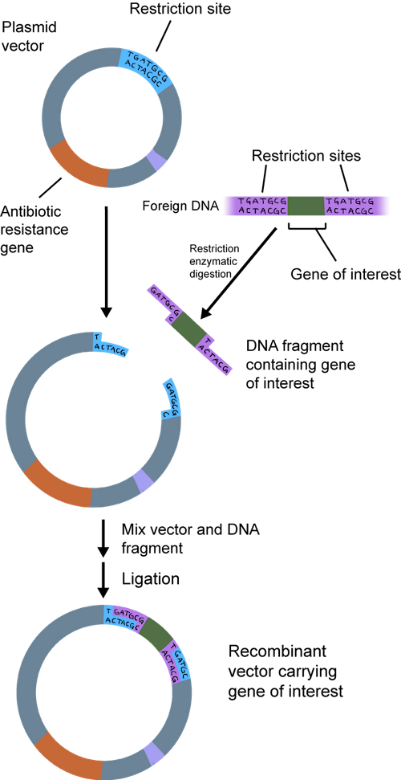
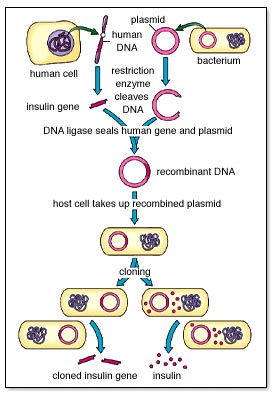
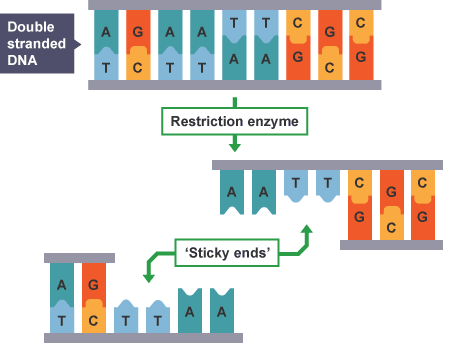
1. Scientists often use PCR to make copies of a DNA sample before it is studied using a technique called gel electrophoresis. During gel electrophoresis, a DNA fingerprint is created. This fingerprint shows a sample of DNA as a pattern of “bands” (lines). An example of a DNA fingerprint is shown to the right. The pattern of bands on a DNA fingerprint differs from person to person, so it can be used for identification purposes.
2. To create a DNA fingerprint, the DNA sample must first be chopped into smaller pieces using enzymes called restriction enzymes. These restriction enzymes recognize specific sequences of nitrogen bases on the DNA called restriction sites. They cut the DNA at these restriction sites. Each person’s DNA has different restriction sites, so different sized pieces (aka fragments) of DNA will be produced for each person’s DNA sample.
3. After the DNA has been cut using the restriction enzymes, the DNA sample is injected into small holes at the end of a rectangular sheet of mesh-like gel made from a polysaccharide (i.e., large carbohydrate) called agarose. The DNA is injected into these holes using a tool called a micropipette (see image to the right). A micropipette is basically just an eyedropper that picks up and drops out small amounts of liquid. The holes in the gel are called wells. A different DNA sample is injected into each well.
4. The gel sits in a plastic box called a chamber. The gel is covered by a buffer solution, which consists of water and salts. Once all the DNA samples have been loaded into the wells, an electrical current is run through the chamber. This makes the well end of the gel negatively charged and the far end of the gel positively charged.
5. Because DNA is negatively charged, the fragments in the wells are attracted to the positive far end of the gel (remember, opposites attract!). The DNA fragments migrate (move) through the mesh-like gel towards the positive end. Smaller fragments are able to fit through the spaces in the mesh more easily, so they move more quickly towards the positive end than larger fragments. When the smallest fragments have reached the far end of the gel, the current is turned off. At this point, the gel can be stained so that the bands of the DNA fingerprint can be visualized. Each band contains many copies of a DNA fragment of a particular size.
6. Because the banding pattern on a DNA fingerprint is unique to each person, DNA fingerprints can be run on samples of DNA (in blood, saliva, etc.) from a crime scene to determine which suspect committed a crime. They can also be used to determine the paternity of a baby. In other words, they can be used to determine if a man’s DNA and a baby’s DNA match up enough for the man to be considered the baby’s biological father.
7. DNA fingerprints can also be used in medicine to determine if a person has a DNA banding pattern characteristic of a genetic (inherited) disease like cystic fibrosis, sickle cell disease, etc.
8. DNA fingerprints can also be used to compare DNA samples from different species.
9. Other methods can be used to determine the full sequence of nitrogen bases within a person’s DNA. The full set of genes or genetic material found in one cell of an organism is called that organism’s genome. Large groups of scientists worked collaboratively over many years to determine the full sequence of bases in the human genome. This major project was called the Human Genome Project.
10. In addition to sequencing the human genome, scientists also determined the locations of major genes on human chromosomes to create a genetic map of the chromosomes. Remember, genes are sequences of bases on DNA that code for the creation of a polypeptide and determine a particular trait within the cell or organism as a whole. The human genome has about 20,500 genes and three billion base pairs.
11. The Human Genome Project was completed in April 2003 and its results were published in the major scientific journal *Nature.* When scientists publish their results in a research article, the article must be submitted to a journal (or multiple journals) for review. A process called peer review is used, in which other scientists evaluate the methods used in the research and question the legitimacy of the results and conclusions. If the article is deemed legitimate, it may be accepted by the journal and published.
12. The potential applications (uses) of the Human Genome Project are wide-ranging. The project can be used to identify genes that can cause genetic diseases when mutated. It can also be used to develop treatments such as gene therapy (discussed later in the notes) that target a particular mutated gene sequence.
13. Scientists are currently working on sequencing the genomes of other species, particularly those that are often used as study organisms in the lab (ex: mice, fruit flies, worms). We can compare human DNA to the DNA of other species to determine similarities and differences. We can use these similarities and differences to determine how closely related we are to other species (more similarities = more closely related). Humans share about 99% of their genome with chimpazees. That does not mean that we evolved from apes! It simply means that we share a recent common ancestor.

**How can DNA be modified?**

1. Scientists have developed techniques to modify/alter/manipulate DNA. Three techniques we are going to be discussing are cloning, the creation of recombinant DNA, and the creation of transgenic organisms.
2. When scientists clone a whole organism, they can use a technique called somatic cell nuclear transfer. This is how scientists cloned Dolly the sheep in 1996.
3. During somatic cell nuclear transfer, a nucleus is removed from a somatic cell (a body cell).
4. This nucleus is then put in an egg cell that has been enucleated (i.e., had its nucleus removed). The egg is then carrying all the DNA from the organism that donated the somatic cell and does not need to be fertilized by a sperm.
5. The egg is then put in the uterus of a surrogate mother and develops into a clone of the somatic cell donor (not the egg donor or surrogate mother).

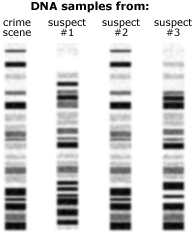
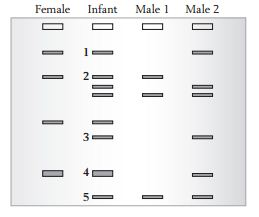
 

1. Scientists can mix DNA from different species. This mixed DNA is called recombinant DNA. Scientists often create recombinant DNA using a small circle of DNA from a bacterium called a plasmid. (The plasmid is separate from the bacterium’s large circular chromosome).
2. Scientists must first extract (remove) the plasmid from a bacterium.
3. Scientists then cut the plasmid DNA using a restriction enzyme. This creates single-stranded “overhangs” on the DNA called sticky ends.
4. Scientists then cut the DNA sequence they would like to insert into the plasmid using the same restriction enzyme. This creates sticky ends that are complementary to the sticky ends on the plasmid DNA.
5. The bases on the sticky ends of the target sequence and plasmid DNA hydrogen bond with each other. They are fully sealed together using the enzyme ligase.
6. An example of a DNA sequence we may want to put in a plasmid is the human insulin gene. Once we have put this gene into the plasmid, we can force bacteria to take the plasmid back in using a calcium chloride (CaCl2) solution and a process called heat shocking. When the bacterium takes the recombinant plasmid in, this is called transformation. Once the plasmid is inside the bacterium, the bacterium can replicate the plasmid DNA and the human insulin gene. It can also transcribe and translate the human insulin gene to create human insulin protein. The human insulin protein can then be extracted (removed) from the bacterium and used to treat diabetes!



1. Scientists can use recombinant DNA to create transgenic organisms, which are organisms with DNA from two or more species. Transgenic organisms are a type of genetically modified organism (or GMO for short). Genetically modified organisms are organisms that have had their DNA altered in some way.
2. An example of a transgenic organism is the spider goat (see image to the right), which is a goat that is able to make spider silk using a gene from a spider. Transgenic crops have also been created, which often contain genes that make them resistant to certain pests and herbicides.
3. It is very difficult to create an adult transgenic plant or animal because the new DNA must be delivered to each cell of the multicellular organism. Often this is done by infecting the organism with a bacteria that carries a recombinant plasmid with the target gene. The genes that make the bacteria harmful to the organism have been removed.
4. This method of using a bacteria (or virus) to deliver genes to the cells of an organism could be used during gene therapy to deliver a healthy version of a gene to a person with a genetic disease.

**Notes Questions**

1. What is the goal of PCR?
2. Why is heat used in PCR?
3. What are primers and why are they used in PCR?
4. What is Taq polymerase, and why is it used in PCR?
5. Why are restriction enzymes used during gel electrophoresis?
6. Why is an electrical current used during gel electrophoresis?
7. Why do smaller fragments of DNA move farther in the gel?
8. What are two possible applications (uses) of DNA fingerprinting?
9. Using the information in the DNA fingerprint to the right, which suspect committed the crime? Explain your answer.
10. Using the information in the DNA fingerprint to the right, which male could be the baby’s father? (Note: On a baby’s DNA fingerprint, each band had to come from either the mother or father. I’m not sure why they have numbered some of the bands on this image!)
11. What were the original goals of the Human Genome Project?
12. What are two possible applications (uses) of Human Genome Project?
13. Explain why a cloned organism created through somatic cell nuclear transfer is identical to the somatic cell donor.
14. Explain how restriction enzymes are used to create recombinant plasmids. Use the term “sticky ends” in your answer.
15. Explain how ligase is used during the creation of a recombinant plasmid.
16. Explain how human insulin protein is created by a bacterium that has a recombinant plasmid containing the human insulin gene.
17. What are transgenic organisms? Provide an example of a transgenic organism.
18. Why is it so difficult to create an adult transgenic organism? How do we do this?
19. What is gene therapy?